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EVALUATION OF THE CHELATING EFFECT OF METHANOLIC EXTRACT OF *CORIANDRUM SATIVUM* AND ITS FRACTIONS ON WISTAR RATS POISONED WITH LEAD ACETATE

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Abstract

Background: The rate of lead poisoning has decreased in recent years due to increased health control in industries that use this metal. However, it is still a public health problem worldwide. The use of various plants with chelating properties has been a topic of research today. In traditional medicine, it is said that *Coriandrum sativum* has chelating properties, but there is no scientific evidence to support this fact. The purpose of this research is to evaluate the chelating effect of methanol extract of coriander and its fractions on Wistar rats intoxicated with lead.
Materials and Methods: In this research, male Wistar rats were poisoned with 50 mg/kg of lead acetate and treated with 50 mg/kg of methanol extract and its fractions. The extract and its fractions were administered to four treatment groups. Positive and negative controls were established. Hemoglobin, hematocrit and lead concentrations were analyzed; liver was evaluated histologically in control and treatment groups.
Results: The methanol extract of coriander presented a LD_{50} >1000 mg/dL. The group administered with the methanol extract showed significant difference in the levels of hemoglobin and hematocrit compared to the negative control group. Lead concentration in treatment groups showed a decrease compared to the positive control. Histological evaluation of tissue showed less damage in groups administered with methanolic extract and its fractions compared to the positive control which presented structural alterations.
Conclusion: Coriander extracts protect liver and lower lead concentration in rats intoxicated with lead in contrast to the positive control group.

Key words: Lead, chelatin effect, natural products, *Coriandrum sativum.*

Introduction

The chelation systems, currently they are an important alternative for the detoxification of xenobiotics given control needs in areas highly contaminated by heavy metals such as lead (Pb). In the Laguna Region (Northern Mexico (25°32'40"N; 103°26'31"W), lead contamination creates a great need to develop such strategies chelation which lead to improved human health. La Laguna region is currently one of the areas with the highest lead contamination in Mexico (Calderón Salinas et al., 1996 a, b; García Vargas et al., 2001; Morán Martínez, 1998, Morán Martínez et al., 2013). The use of several plants with chelation properties is one of the principal research items in present days. Garlic extract alleviates Pb-induced neural, hepatic, renal and haematic toxicity in rats and prevents Cd-induced mitochondrial injury and apoptosis in tissue culture models (Sharma et al., 2010; Sadeghi et al., 2013; Murugavel et al., 2007; Lawal and Ellis, 2011). Ginger and onion have similar antioxidant capacities with garlic, and using these food ingredients as supplement prevents Pb-induced renal and developmental toxicity as well as Cd-induced gonadotoxic and spermiotoxic effects in rats (Reddy et al., 2011; Ola-Mudathir et al., 2008; Farag et al., 2010). The flavonoids and phenols in curry leaves function as antioxidants and as potential chelators, which prevent Cd-induced cardiac toxicity (Mitra et al., 2011). Fruits such as grapes are also effective against Cd toxicity (Pires et al., 2013). Besides the function of vitamins and essential metals in grapes, the abundant polyphenols such as anthocyanins may also alleviate oxidative stress caused by Cd and Pb toxicity. Tomato is regarded as one of the most powerful

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natural antioxidants (Shi and Maguer, 2000) and can prevent renal toxicity induced by rats exposure to Pb (Salawu et al., 2009). Moreover, tomato has been reported to produce metal chelating proteins and phytochelatins when exposed to heavy metal ions (Tito et al., 2011; Steffens et al., 1986). In fact, the oral administration of tomato significantly reduces the accumulation of heavy metals (Cd, Pb and Hg) in the liver of rats (Nwokocha et al., 2012). To develop new natural alternatives with biological activities and reduced secondary effects, we evaluate the chelating effect of *Coriandrum sativum* extract on Wistar rats administrated with lead acetate. *C.sativum* L., commonly known as "Coriander", is an annual small plant like parsley which originated around 1550 BC, and is one of the oldest spice crops in the world (Coskuner and Karababa, 2007). It belongs to Apiaceae family in the order of Apiales that contain about 300 genera and more than 3000 species (Asgarpanah et al., 2012). Coriander leaves are widely used for folk medicine as carminative, spasmolytic, digestive, and galactagogue. It is stable and retains its pleasant odour longer than any other oil of its class (Eikani et al., 2007). In traditional medicine, *Coriandrum* possesses strong chelating properties, but it does not have enough scientific evidence to support this (Gurib, 2006). The detection of alterations in the hematopoietic system contributes to the diagnosis of the rats exposure to lead (Souza and Tavares, 2009). Despite attempts of reducing the animals' exposure to this metal, there are still some cases of severe lead toxicity (Saper et al., 2004). Chelating agents are substances that bind to heavy metals which flow through the bloodstream forming water soluble and non-toxic compounds that are released by urine and bile (Fontana et al., 2013). The treatment used for heavy metal intoxication has improved with time. Some studies in mice and rats intoxicated with different concentrations of lead and treated with *C. sativum* show very encouraging results chelation and reduction poisoning in these animal models (Aga et al., 2001; Sharma et al., 2010; Velaga et al., 2014). However, we need to find a natural treatment less invasive and performs like chelating agents. The goal of this investigation is to reduce treatment cost and any secondary effect that can affect public health (Flora, 2003). The aim of this research was to evaluate the chelating effect of methanol extract of coriander and its fractions on Wistar rats intoxicated with lead.

Materials and Methods

Ethical and legal aspects of the study

Experiments were carried out in accordance with the International Guidelines on the Appropriate Use of Experimental Animals, and according to Mexican Norm NOM-062-ZOO-1999 on the Technical Specifications for Production, Care and Use of Laboratory Animals (SAGARPA, 2010). The experiments were maintained by an expert veterinarian with professional license: 4807528. The protocol was approved by the Bioethical Committee of the Faculty of Medicine Autonomous University of Coahuila, Torreon Campus, Coahuila, Mexico. The number of approval by the Secretaría de Salud and Comisión Nacional de Bioética in Mexico was:
CONBIOETICA07CEI00320131015.

Collection of plants

About 5 kg of leaves and stalks of *C.sativum* were collected in the rural area of Ciudad Juarez Durango in the North of Mexico. It is located on latitude 25.5° and longitude -103.58° (API Google Maps), with a height of 1,140 m above sea level. Guidelines for Good Practice Plant Collection proposed by World Health Organization (WHO, 2003) were used to collect tested plants. A voucher specimen (number 012-2014) was deposited at the herbarium of the Universidad Autónoma Agraria Antonio Narro, Campus Torreón.

Extraction and Preparation of *Coriandrum sativum* Fractions

The shade-dried and powdered leaves-stalks of Coriandrum sativum were macerated in methanol. Adding 30 g of dry grinded material soaked with 300 mL of methanol (analytical grade, 99.98% purity, JT Baker, USA) in an Erlenmeyer flask (Payrex, USA). The extract was filtered using Whatman filter paper No. 1, the organic solvent from filtrate was removed using a Rotavapor (Buchii-215®, USA). Crude extract was completely dried in a vacuum oven at temperature lower than 50°C for 7 days (Benchmark Scientific Mini Incubator, USA). Dried extracts were stored at 4°C, in amber vials until use (Nostro et al., 2000; Navarro et al., 2006).

Extract fraction

With the methanolic extract, was obteined the hexane fraction by maceration in a proportion of 1:10, for dose solvents by extracts. Hexane soluble fraction was filtered and distilled in a rotavapor (Rotavapor Buchii-215®, USA) to evaporate the solvent. After the highest content of hexane-soluble compounds was obtained, the part not solubilized with hexane was macerated with chloroform, leading to the realization of the following fractions: Hexane soluble fraction, chloroform soluble fraction and chloroform non-soluble fraction.

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Toxicity test with *Artemia salina* nauplii

For the toxicity test, 0.1 g eggs of *A.salina* (Brine Shrimp Eggs® San Francisco Bay Brand, INC) was incubated in artificial sea water in a dark container. The container is divided by a middle wall and has a space of 2 mm at the bottom. The pH was adjusted to 7.8, and the container was kept under artificial white light and oxygenation. 48 h later, the hatched larvae called nauplii were taken with a Pasteur pipette and transferred to another container. They were kept under light, oxygen and temperature of 22 to 29°C for 24 h. 100 µL of seawater containing 10 nauplii per well plus 100 µL of vegetal extract (10. 50, 250, 500 and 1,000 µg/mL) were placed on a microplate of 96 wells. Four replicates were used as positive control potassium dichromate (400 ppm concentration) and sea water was used as negative control. After 24 h, with the help of a stereoscopic microscope, the total count of live and dead nauplii per dose was done. Probit method was used to determine the LD50 (Bastos *et al*., 2009, Déciga *et al*., 2007).

Phytochemical analysis for partial identification of the extract components

The qualitative phytochemical investigations of the crude extract were carried out using Galiste (1997) and Ali (1991) methods (Table 1).

Table 1: Phytochemical analysis for partial identification of the extract components.

Functional groups	*Coriandrum sativum*	Functional groups	*Coriandrum sativum*
Coumarins	+	**Tannins**	
Steroids	-	**Catequic**	-
Terpenes	+	**Pirogalic**	+
Anthocyanins	-	**Alkaloids**	-
Saponins	-	**Flavones**	+
Flavonoids	-	**xanthones**	-

Experimental design

Male Wistar rats (220-230 g weigth) were maintained under controlled conditions: 12/12 h cycle light/darkness, temperature of 25 ± 3°C, relative humidity of 35 to 60%. They were given Harlan food and water *ad libitum* until the end of the experiment. Six groups were formed randomly with five animals in each.

For the administration of the two treatments in all groups: lead poisoning and the methanolic extract of *C. sativum* the exposure time was 21 days.

Group I: 30 mg $kg^{-1}$of Lead acetate was administered intraperitoneally three times weekly. 50 mg kg^{-1} *C. sativum* methanolic extract was administered daily in a gastric tube.

Group II: 30 mg kg^{-1} of Lead acetate was administered intraperitoneally three times weekly. *C.sativum* hexanic fraction of 50 mg kg^{-1} was administered daily in a gastric tube.

Group III: 30 mg kg^{-1} of Lead acetate was administered intraperitoneally three times weekly. *C.sativum* chloroformic fraction of 50 mg kg^{-1} was administered daily in a gastric tube.

Group IV: 30 mg kg^{-1} of Lead acetate was administered intraperitoneally three times weekly. *C.sativum* non-soluble chloroformic fraction of 50 mg kg^{-1} was administered daily in a gastric tube.

Group V: (Negative control): saline solution was administered intraperitoneally three times weekly. Distilled water was administered daily using a gastric tube.

Group VI: (Positive control): 30 mg kg^{-1} of lead acetate was administered intraperitoneally three times weekly. EDTA of 50 mg kg^{-1} was administered daily in a gastric tube.

Blood analysis

Hemoglobin and hematocrit determination

After 21 days of oral administration of the extracts to the treatment and control groups, the animals were sacrificed by cervical dislocation (This sacrifice method is in according with Mexican Norm NOM-O62 ZOO-1999). This Norm establish that animals less than 250 g body weight is allowed the cervical dislocation). 4 mL of blood was taken by cardiac puncture and placed in an EDTA vacutainer tube for blood count with a COULTER-T-860 analyzer (Beckman Coulter, CA, USA). The hematocrit was calculated (Red blood cell count/mm^3 x MCV (Medium Corpuscular Volume).

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Lead serum determination

According to the manufacturer's instruction (application note AA3004), Buck Scientific's 210VGP Atomic Absorption Spectrophotometer was used to determine lead in the blood (CT, USA).

Histological evaluation of liver

Liver samples were processed by conventional histological techniques until they were included in paraffin blocks (Paraplast, Surgipath, Leica Biosystems Ricmond, Inc, IL, USA). In the microtome (Leica 1512, Germany) histological sections of 5micron thickness stained with hematoxylin-eosin were obtained for histological evaluation using light microscope (Velab Microscopes ®).

Morphometric analysis

Consecutive fields with oil immersion objective (100x) were analyzed subsequently differentiating normal hepatocytes showing the changes in the three areas described for liver acinus were quantified, this was performed in triplicate. Mean value and standard deviation (SD) were obtained and analyzed statistically with Student t test for a value of $p<0.05$ significance.

Results and Discussion

The results obtained in this study are promising due to the treatment groups show a decreased in lead serum levels with respect to the control groups. Also, there is observed an hepatoprotective effect in rats administered with *C sativum* extracts. Table 2 shows fractions of *C. sativum* extract where the methanol extract is considered as 100%. The eluent used in the process of maceration was methanol. It was selected for the following reasons: its high polarity, the type of molecule responsible for it is unknown and is a crude extract with the greatest number of bioactive components. Subsequently, the extract was subjected to fractionation and its activity as chelating agent was tested, as proposed by Rondina et al., (1991). To evaluate the biological activity of *C. sativum* as a chelating agent and to find a natural treatment that is less invasive than synthetic products, we developed *A. salina* lethality to determine the LD_{50} This was done because this technique has a potential correlation with specific toxicity tests (McLaughlin, 1998) and is based on the suggestion of WHO (2002) for natural products safety. In this paper, we found a LD_{50}> 1,000 mg/mL for *C. sativum* methanol extract. This indicates that the extract is not toxic due to a high dose is needed in order to kill the 50 percent of organisms according to Lagarto-Parra et al., (2001) and the scale of Déciga-Campos (2010). Based on these criteria, it can be said that *C. sativum* is promising, due to its chelating ability and has no reports of toxicity since its use as food from ancient times till date. The phytochemical screening of this study shows the presence of flavones, terpenes, pyrogallic tannins and coumarins (Figure 2), similar to the report given by Rajeshwari and Andallu (2011), other authors attribute antioxidant activity to coumarins and flavonoids (Enayde et al., 2005; Wong and Kitts, 2006). According to Patrick (2006), antioxidant therapy has great opportunity in patients poisoned with this metal. Lead can promote the generation of reactive oxygen species, and affect antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase among others. These enzymes are essential part of the antioxidant system and they are responsible for the removal of reactive species or free radicals such as lead (Nordberg and Arnér, 2001). However, when there is a prolonged lead poisoning activity, these enzymes are affected to the extent of decreasing their efficiency. The compounds in *C. sativum* can strengthen the antioxidant system. When they react with lead, they are not metabolized, thereby decreasing the serum levels of this metal. Figure-1 shows means of the hematocrit concentrations in treatment and control groups. Significant difference was observed in groups 2 and 3 compared to the negative group ($p<0.05$). With respect to the positive control group, there was no significant difference except in group 1 ($p<0.05$). However, in groups 2, 3 and 4, a small increase in hematocrit levels is observed compared to the lead poisoning group or positive control ($p<0.05$). This could be due to the recovery of intoxicated groups and those treated with *C. sativum* fractions. These results agree with those reported by Alcazar-Montenegro et al., (2000) where inpatients with various liver diseases, hemoglobin and hematocrit significantly improved due to the administration of thiamine pyrophosphate. Blood levels of hemoglobin (Figure-2) in treatment groups show a significant decrease compared to the negative control, but are above the positive control group ($p<0.05$). These are encouraging results since several researchers have shown that intoxication with this metal causes anemia which is reflected in a decrease of hemoglobin (Golalipour et al., 2007). In a study done by Ibrahim et al., (2012), they established that hemoglobin decreased by 25% in rats intoxicated with lead. In this investigation, we observe a recovery effect in groups 1, 2 and 3 above the 25 % according with the observed by Golalipour et al., (2007). Figure-3 shows the mean concentration of lead in treatment and control groups. In group 1, there was increase in the concentration of lead compared to the other treatment groups and the negative control groups. On the other hand, group 4 showed a significant decrease with respect to group 6 (positive control) ($p<0.05$). In groups 2 and 3, a decrease of lead in blood is observed; even the statistical analysis did not show a significant difference ($p>0.05$). Calderon et al., (2012) conducted a study where they used 60 Wistar rats, with average weight of 185 g divided into five groups. They established that different groups were poisoned with lead and administered with different treatments like N-acetylcysteine (NAC), Methionine (MET), NAC + MET. The results showed that NAC lowered blood lead levels by 23% ($p<0.05$). The combination of NAC+MET lowered blood lead levels by 41% ($p<0.001$). It is interesting to note that the results are very similar to those observed in the present study. The treatment groups were significantly different from the control groups. In our study, the

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group that was administered with EDTA did not show a decrease in lead levels. This can be attributed to the mode of administration. In this study, EDTA was administered in gastric tube but in other studies, it was done intraperitoneally, with greater efficiency (Reckziegel et al., 2011; Sánchez et al., 2002; Flora et al., 1995; Bankowaska and Hine, 1985). Despite this, the results obtained from our treatment groups were very satisfactory (Figure-3). On the other hand, other similar rodent studies as described by Aga et al., 2001, show a significant reduction of lead and reduced delta aminolevulinic acid post treatment. Similarly, Sharma et al., 2010, they found a protective effect against oxidative stress induced by lead in mouse testes. In positive control group administered with EDTA, histopathology shows that lead produced histological damage characterized by deformation of the radial arrangement of hepatocytes, sinusoidal decrease, increase of Kupffer cells, pyknotic nuclei, vacuoles, bi-nucleated cells and anisonucleosis (Figure-4B). Porru and Alessio (1996) have reported that EDTA prevents Pb toxicity, however, it can cause renal toxicity (at the proximal tubule, particularly), especially during repeated high dose treatment (above 75 mg/kg) and in subjects with previous history of kidney damage. Some of these changes were reversed to a greater or lesser extent depending on the fractions administered. In liver sections of rats exposed to lead acetate (30 mg/kg) and treated with the chloroformic non-soluble fraction (Figure-4F), there were lobules of variable sizes, decrease in the number of Kupffer cells per lobule, nucleus well defined and few bi-nucleated cells. In the group administered with hexane fraction (Figure-4D), there was reduction in the size of sinusoids and fragments of vasocongestion; however, there was no improvement in hepatocyte radial arrangement with fewer Kupffer cells by sinusoid, no binucleated cells and pyknosis. Group treated with methanol extract had better radial arrangement of hepatocytes, some of their nuclei were slightly hyperchromatic and had more than one nucleolus. With the administration of methanol extract lobules, there were several nucleoli and improvement of radial arrangement of hepatocytes (Figures-4C and -4F). Histologically, the changes caused by lead in liver agree with the results of Shalan (2006) where there was an improvement in tissues of rats intoxicated with lead and administered with vitamin C. Also, Sharma et al., (2010) evaluated the effect of oral administration of *Allium sativum* in male mice intoxicated with lead. They observed an improvement in the tissue of mice administered with *A. sativum.* This is attributed to the antioxidant potential of *A. sativum*. Both studies are very similar to our results, where the hepatoprotective activity of *A. sativum* is attributed to antioxidant compounds (Shalan, 2006; Sharma et al., 2010). In relation to the alterations produced in the architecture of the lobe as well as cellular and nuclear level, these results are important because they allow a decrease in tissue damage on treatment groups compared to the EDTA group. Treatment of lead poisoning is based on the use of chelating compounds, which eliminate the metal from organism by forming coordinated compounds. Lead causes free radicals and dejection of cellular antioxidant defense system. Currently, various antioxidants from plants are evaluated. Plant extracts have potential chelation effect on some metal, preventing or reversing the damage caused by lead in various tissues (Shalan et al., 2005; Shang et al, 2009; Wang et al., 2006; Patra et al., 2001). In the present study, we found that methanolic extract and its different fractions used individually led to diverse results in relation to the histological alterations caused by lead sub-acute intoxication. In the group treated with methanol extract, it was observed that the extract has antioxidant ability, thus reducing lead accumulation in various organs by exerting a synergistic or enhancing chelating effect (Shalan et al., 2009; Reddy et al., 2010).

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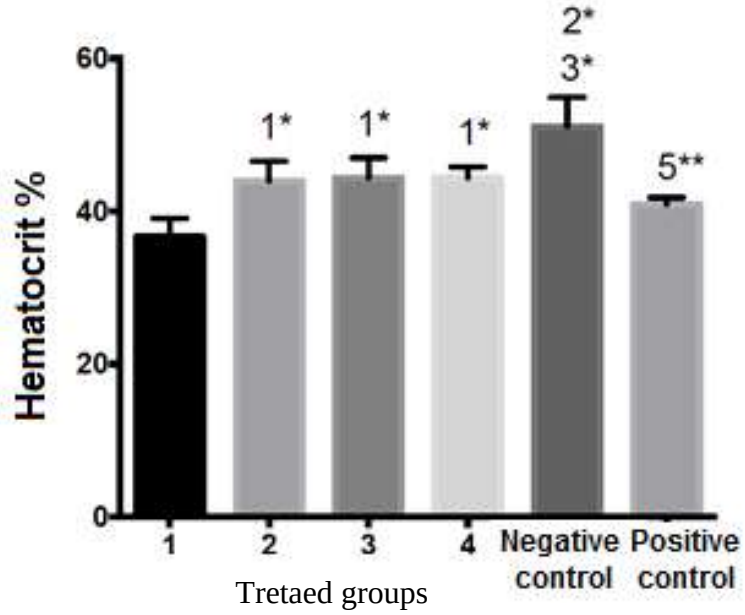

Figure 1. Mean correlation of hematocrit on treatment, negative and positive control groups. (*p<0.05 negative control *vs* groups 2 and 3; *p<0.05 groups 2, 3 and 4 *vs* 1 and **p<0.01 positive control *vs* negative control).

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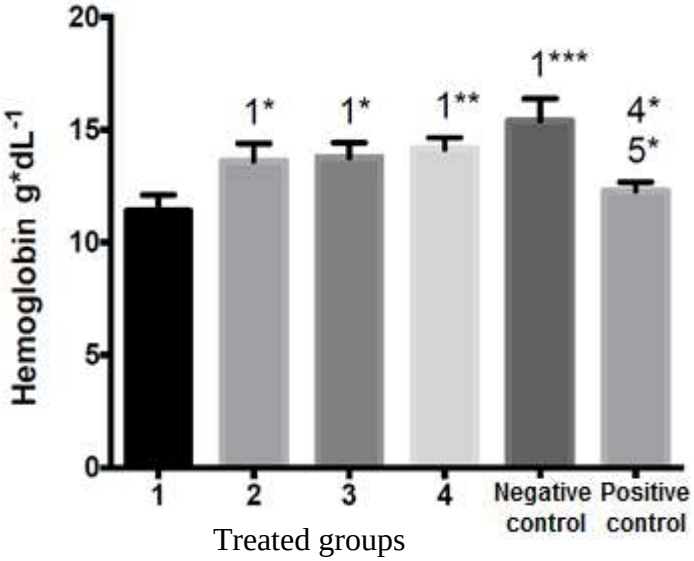

Figure 2. Mean correlation of hemoglobin on treatment, negative and positive control groups. *p<0.05;**p<0.01; ***p<0.001

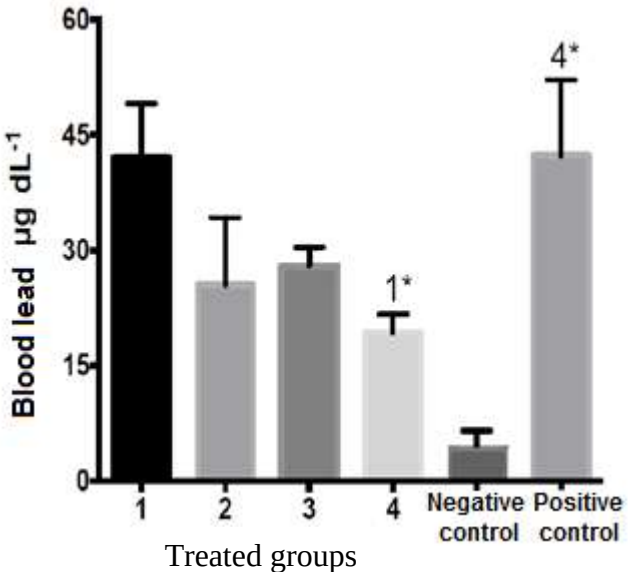

Figure 3. Mean concentration of lead on treatment and control groups. (*p<0.05 positive control *vs* group 4; *p<0.05 group 4 *vs* group 1).

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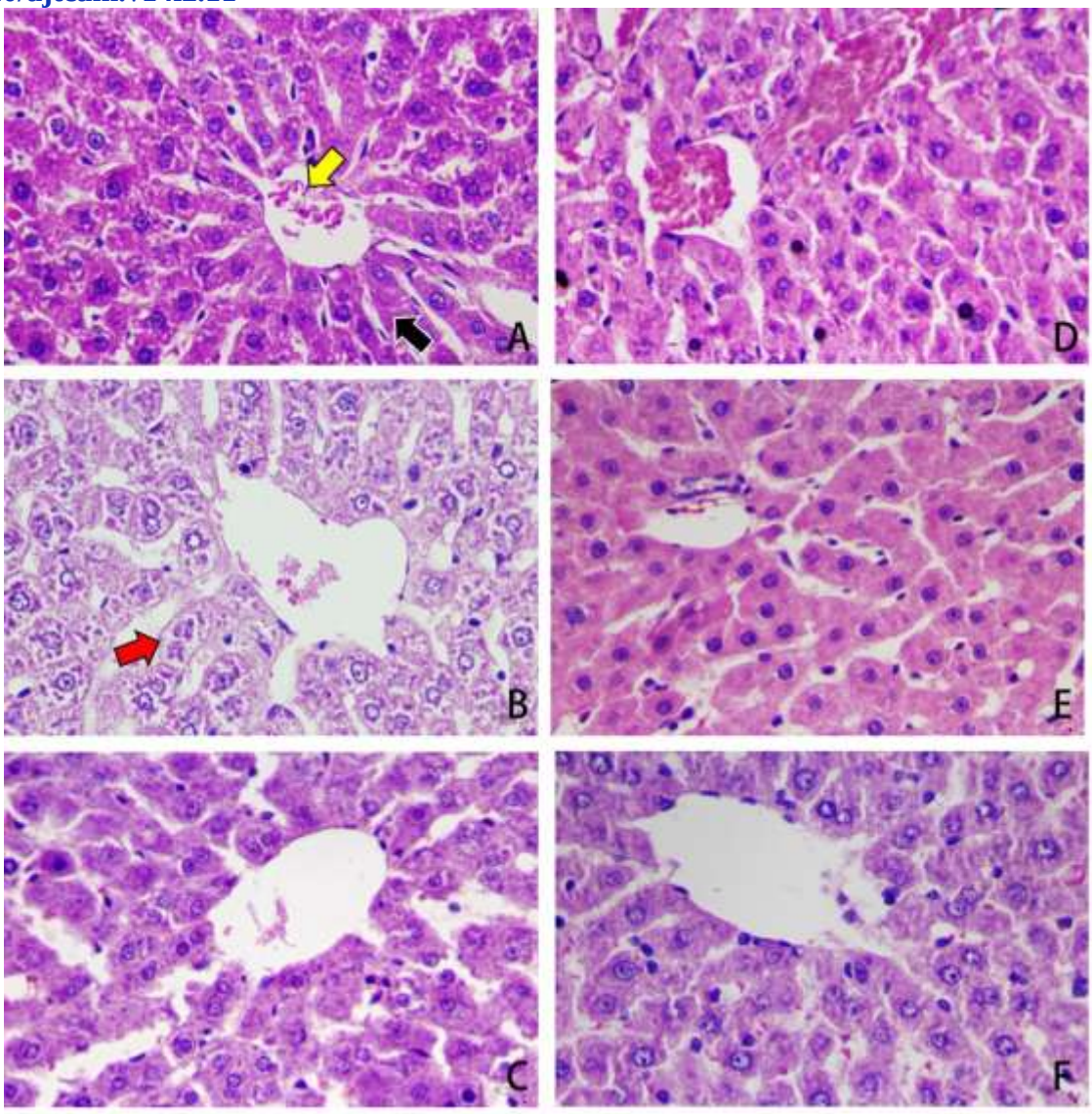

Figure 4. Liver micrograph of control and intoxicated rats. Negative control group (A) in which central vein is observed (yellow arrow) and hepatocytes (black arrow) of normal histological features. In the positive control group (B) an evident generalized vacuolization is observed in the cytoplasm of hepatocytes (red arrow). In experimental groups 1, C. sativum methanolic extract (C), 2 C. sativum hexanic extract (D), 3 C. sativum chloroformic fraction (E) and 4 C. sativum chloroformic non-soluble fraction (F) were not observed evident, except for the presence of some areas of vascular congestion and some hyperchromatic nuclei, especially in group C. sativum extract hexanic histological alterations. Paraffin embedded. Staining hematoxylin and eosin. 40X.

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Conclusion

The administration of the methanol extract and its fractions produced a good significant variation in most of the evaluated tests. However, some histological alterations in variable degrees persist, suggesting that treatment with these antioxidants may be useful to partially reverse alterations from exposure to lead.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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References

1. Aga, M., K. Iwaki, Y. Ueda, S. Ushio, N. Masaki, S. Fukuda, T. Kimoto, M. Ikeda, M. Kurimoto. (2001). Preventive effect of Coriandrum sativum (Chinese parsley) on localized lead deposition in ICR mice. J. Ethnopharmacol. 77(2-3):203-8.
2. ATSDR (Agency for Toxic Substances and Disease Registry) (2007). Toxicological profile for lead. (Draft for Public Comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
3. Alcázar-Montenegro, H., S. Alcázar-Leyva, E. Aguirre-Benítez, N. Alvarado-Vásquez, R.M. Rivera-López, A. Gámez-Murrieta and M.T. Benítez-Rodríguez. (2000). Disfunciones hepáticas tratadas con pirofosfato de tiamina. Resultados clínicos y de laboratorio. Bioquimia. 25(2):45-48.
4. Ali, T.H.M. (1991). Estudios sobre lavanda multifida L." Tesis doctoral. Inéd. Facultad de Farmacia, Universidad de granada.
5. Almeida-Alves, T.M., A. Fonseca-Silva, M. Brandão, T.S. Mesquita-Grandi, E.F. Smânia, A.S. Júnior and C.L. Zani. (2000). Biologicalscreening of brazilian medicinal plants. Memórias do Instituto Oswaldo Cruz. 95:367-373.
6. Anderson, J.E., C.M. Goetz, J.L. McLaughlin and M. Suffness. (1991). A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor prescreens. Phytochem. Analysis. 2:107-111.
7. Asgarpanah, J., M.G. Dadashzadeh, M. Ahmadi, R. Ranjbar and M. Safialdin-Ardebily. (2012). Chemistry, pharmacology and medicinal properties of Heracleum persicum Desf. Ex Fischer: A review. J. Med. Plants Res. 6(10):1813-1820.
8. Bankowska, J. and C. Hine. (1985). Retention of lead in the rat. Archives Environ. Contam. Toxicol. 14:621-629.
9. Bastos, M.L.A., M.R.F. Lima, L.M. Conserva, V.S. Andrade, E.M.M. Rocha and R.P.L. Lemos. (2009). Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheriatuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. Annals Clin. Microbiol. Antimicrob. 8 (16):1-6.
10. BuckScientific según la Nota de aplicación #AA3004
11. Calderón-Salinas, J.V., C. Hernández-Luna, B. Valdez-Anaya, M. Maldonado-Vega and A. Lopez-Miranda. (1996a). A Evolution of lead toxicity in population of children. Hum. Exp. Toxicol. 15: 376-382.
12. Calderón-Salinas, J.V., B. Valdéz-Anaya, M. Zúñiga-Charles and A. Albores-Medina. (1996b). Lead exposure in a population of Mexican children. Human Exp. Toxicol; 15: 305-311.
13. Coskuner, Y. and E. Karababa. (2007). Physical properties of coriander seeds (*Coriandrum sativum* L.). J. Food Engin. 80(2):408-416.
14. Dahanukar, S.A., R.A. Kulkarni and N.N. Rege. (2000). Pharmacology of medicinal plants and natural products. Indian J. Pharmacol. 32(4):81-118.
15. De Almeida, M.E., F.J. Mancini and N.G. Barbosa. (2005). Characterization of antioxidant compounds in aqueous coriander extract (*Coriandrum sativum* L.). LWT-Food Sci. Technology. 38:15-19.
16. Déciga-Campos, M., I. Rivero-Cruz, M. Arriaga-Alba, G. Castañeda-Corral, G.E. Angeles-López, A. Naverrete and R. Mata. (2007). Acute toxicity and mutagenic activity of Mexican plants used in tradicional medicine. J. Ethnopharmacol. 110:334-342.
17. Farag, A.G., M.E. Elhalwagy and H.E. Farid. (2010). Effect of ginger supplementation on developmental toxicity induced by fenitrothion insecticide and/or lead in albino rats. Pestic. Biochem. Physiol. 97:267–274.
18. Flora, J.S., M. Pande and A. Mehta. (2003). Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiolchelators in the treatment of chronic lead intoxication. Chem. Biol. Inter. 145:267-280.
19. Fontana, D., V.M. Lascano, N. Solá, S. Martinez, M. Virgolini and M.R. Mazzieri. (2013). Intoxicación por plomo y su tratamiento farmacológico. Revista de Salud Pública. (XVII)1:49-59.
20. Galisteo, M.M.M., T. Rosmarinus and M. Huber-Morath. (1997). Estudio farmacognóstico. Tesis doctoral inéd. Facultad de farmacia, Universidad de Granada.

doi:10.21010/ajtcam.v14i2.11

21. García-Vargas, G.G., M. Rubio-Andrare, L.M. Del Razo, V. Borja-Aburto, E. Vera-Aguilar and M.E. Cebrian. (2001). Lead exposure in children in smelter community in Region Lagunera, Mexico. J. Toxicol. Environ. Health. 62: 417-429.
22. Golalipour, M.J., D. Roshandel, G. Roshandel, S. Ghafari, M. Kalavi and K. Kalavi. (2007). Effect of lead intoxication and D-penicillamine treatment on hematological indices in rats. Int. J. Morphol. 25(4):717-722.
23. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol. Aspects Med. 27(1):1-93.
24. Joon, P.H., S.J. Lee, Y. Song, S.H. Jang, Y.G. Ko, S.N. Kang, B.Y. Chung, H.D. Kim, G.S. Kim and J.H. Cho. (2014). Schisandrachinensis Prevents Alcohol-Induced Fatty Liver Disease in Rats. J. Med.Food. 17(1):103-110.
25. Lagarto-Parra, A., R. Silva-Yhebra, I. Guerra Sardiñas and L. Iglesias Buela. (2001). Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD_{50}) in mice in order to determine oral acute toxicity of plant extracts. Phytomedicine. 8(5):395-400.
26. Lawal, A.O. and E.M. Ellis. (2011). The chemopreventive effects of aged garlic extract against cadmium-induced toxicity. Environ. Toxicol.Pharmacol.32:266–274.
27. Massele, A: Y. and C.M. Nshimo. (1995). Brine shrimp bioassay for biological activity of medicinal plants used in traditional medicines in Tanzania. East Afr. Med. J. 72:661-663.
28. McLaughlin, J.L., C. Chang and D.L. Smith. (1998). Simple bioassay for the detection and isolation of bioactive natural products. Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Pardue University, West Lafayerre, IN 47907, USA.
29. Meyer, B.N., N.R. Ferrigni, J.E. Putman, L.B.Jacobsen, D.E. Nichols and J.L. McLaughlin. (1982). Brine Shrimp: a convenient general bioassay for active plant constituents. Plant Med. 45:31-34.
30. Mitra, E., A.K. Ghosh, D. Ghosh, D. Mukherjee, A. Chattopadhyay, S. Dutta, S.K. Pattari and D. Bandyopadhyay. (2012). Protective effect of aqueous Curry leaf (Murrayakoenigii) extract against cadmium-induced oxidative stress in rat heart. Food Chem. Toxicol. 50:1340–1353.
31. Molina-Salinas, G. and S. Fernández-Said. (2006). A modified microplate cytotoxicity assay with brine shrimp larvae (Artemia salina). Pharmacologyonline. 3:633-38.
32. Morán-Martínez, J. (1998). Effects of environmental lead on sperm motility in men. International Conference on Reproductive Health. Bombay, India; 332.
33. Morán-Martínez, J., P. Carranza-Rosales, M. Morales-Vallarta, J.A. Heredia-Rojas, S. Bassol-Mayagoitia, N.D. Betancourt-Martínez and R.M. Cerda-Flores. (2013). Chronic environmental exposure to lead affects semen quality in a Mexican men population. Iran J. Reprod. Med. 11: 4: 267-274.
34. Murugavel, P., L. Pari, S.L. Sitasawad, S. Kumar and S. Kumar. (2007). Cadmium induced mitochondrial injury and apoptosis in vero cells: Protective effect of diallyltetrasufide from garlic. Int. J. Biochem. Cell Biol. 39:161–170.
35. Navarro, G.V., G. Rojas, G.L. Zepeda, M. Avilés, M. Fuentes and A.J.E. Herrera. (2006). Antifungal and Antibacterial Activity of Four Selected Mexican Medicinal Plants. Pharm. Biol. 44(4):297-300.
36. Nava-Ruíz, C. and M. Méndez-Armenta. (2011). Efectos neurotóxicos de metales pesados (cadmio, plomo, arsénico y talio). Arch. Neurocien. (Mex). 16(3):140-147.
37. Nieto, I., A. Salama, J. Cataño and C. Chegwin. (2008). Determinación de la toxicidad de Pleurotusostreatus, Pleurotuspulmonarius y Paxillusinvolutus sobre *Artemia salina*. Revista Iberoamericana de Micología. 25:186-187.
38. Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. SAGARPA.
39. Nostro, A., M.P. Germano, V. D'Angelo, A. Marino and M.A. Cannatelli. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol. 30:379-384.
40. Nwokocha, C.R., M.I. Nwokocha, I. Aneto, J. Obi, D.C. Udekweleze, B. Olatunde, D.U. Owu and M.O. Iwuala. (2012). Comparative analysis on the effect of Lycopersiconesculentum (tomato) in reducing cadmium, mercury and lead accumulation in liver. Food Chem. Toxicol. 50:2.
41. Ola-Mudathir, K.F., S.M. Suru, M.A. Fafunso, U.E. Obioha and T.Y. Faremi. (2008). Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. Food Chem. Toxicol. 46:3604–3611.
42. Patra, R.C., D. Swarup and S.K. Dwivedi. (2001). Antioxidant effects of alpha tocopherol, ascorbic acid and l-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicology. 162(2):81-88.
43. Pires, V.C., A. Gollücke, D.A. Ribeiro, L. Lungato, V. D'Almeida and O. Aguiar. (2013). Grape juice concentrate protects reproductive parameters of male rats against cadmium-induced damage: A chronic assay. Br. J. Nutr. 110:2020–2029.
44. Porru, S. and L. Alessio. (1996). The use of chelating agents in occupational lead poisoning. Occup. Med. 46:41–48.
45. Ramos, W., C. Galarza, G. Ronceros, F. de Amat, M. Teran, L. Pichardo, D. Juarez, R. Anaya, A. Mayhua, J. Hurtado and A.G. Ortega-Loayza. (2008). Noninfectious dermatological diseases associated with chronic exposure to mine tailing in a Peruvian district. Br. J. Dermatol. 159(1):169-74

doi:10.21010/ajtcam.v14i2.11

46. Reckziegela, P., V.D. Tironi, D. Benvegnúa, N. Boufleura, B.R.C. Silva, H.J. Segatc, C.S. Pasec, C.M. Moreira dos Santos, F.E.M. Moraes and B.M. Escobar. (2011). Locomotor damage and brain oxidative stress induced by lead exposure are attenuated by gallic acid treatment. Toxicology Letters. 203(1):74–81.
47. Reddy, S.Y., R. Pullakhandam, K.B. Dinesh. (2010). Thiamine reduces tissue lead levels in rats: mechanism of interaction. Biometals. 23(2):247-253.
48. Reddy, Y.A., M. Chalamaiah, B. Ramesh, G. Balaji and P. Indira. (2011). Ameliorating activity of ginger (Zingiberofficinale) extract against lead induced renal toxicity in male rats. J. Food Sci. Technol. 1:1–7.
49. Rondina, R., P. Palacios, R. Filip and J. D Coussio. (1991). Fraccionamiento de Extractos Vegetales por Extracción Líquido/Sólido Utilizando un Soporte Diatomáceo Inerte. Acta Farm. Bonaerense. 10(1):49-5.
50. Sharma, V., L. Kansal and A. Sharma. (2010). Prophylactic efficacy of *Coriandrum sativum* (Coriander) on testis of lead-exposed mice. Biol**.** Trace Elem**.** Res.136(3):337-54.
51. Sadeghi, A., A.E. Bideskan, F. Alipour, A. Fazel and H. Haghir. (2013). The Effect of ascorbic acid and garlic administration on lead-induced neural damage in rat offspring's hippocampus. Iran. J. Basic Med. Sci. 16:157–164.
52. Salawu, E.O., A.A. Adeleke, O.O. Oyewo, E.A. Ashamu, O.O. Ishola, A.O. Afolabi and T.A. Adesanya. (2009). Prevention of renal toxicity from lead exposure by oral administration of Lycopersiconesculentum. J. Toxicol. Environ. Health Sci. 1:22–27.
53. Sánchez-Fructuoso, A.I., J. Blanco, M. Cano, L. Ortega, M. Arroyo, C. Fernández, D. Prats and A. Barrientos. (2002). Experimental lead nephropathy: Treatment with calcium disodium ethylenediaminetetraacetate. Am. J. Kidney Dis. 40(1):59–67.
54. Saper, R.B., S.N. Kales, J. Paquin, M.J. Burns, D.M. Eisenberg, R.B. Davis and R.S. Phillips. (2004). Heavy metal content of ayurvedic herbal medicine products. J. Am. Med. Assoc. 292(23).
55. Shalan, M.G., M.S. Mostafa, M.M. Hassouna, S.E. El-Nabi and A. El-Refaie. (2005). Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology. 206(1):1-15.
56. Shan, G., T. Tang and X. Zhang. (2009). The protective effect of ascorbic acid and thiamine supplementation against damage caused by lead in the testes of mice. J. Huazhong Univ. Sci. Technol. 29(1):68-72.
57. Sharma, V., A. Sharma and L. Kansal. (2010). The effect of oral administration of *Allium sativum* extracts on lead nitrate induced toxicity in male mice. Food Chem. Toxicol. 48:928–936.
58. Shi, J. and M.L. Maguer. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. Crit. Rev. Food Sci. Nutr. 40:1–42.
59. Souza, A.M. and C.F.F. Tavares. (2009). Chumbo e anemia. Medicina (RibeirãoPreto). 42(3):337-340
60. Steffens, J., D. Hunt and Williams. (1986). Accumulation of non-protein metal-binding polypeptides (gamma-glutamyl-cysteinyl) n-glycine in selected cadmium-resistant tomato cells. J. Biol. Chem. 261:13879–13882.
61. Steinke, D.R., W. Majak, T.S. Sorensen, M. Parvez. (2008). Chelation of molybdenum in Medicago sativa (alfalfa) grown on reclaimed mine tailings. J. Agric. Food Chem. 56(13):5437-42.
62. Tito, A., A. Carola, M. Bimonte, A. Barbulova, S. Arciello, F. de Laurentiis, I. Monoli, J. Hill, S. Gibertoni and G. Colucci. (2011). A tomato stem cell extract, containing antioxidant compounds and metal chelating factors, protects skin cells from heavy metal-induced damages. Int. J. Cosmet. Sci. 33:543–552.
63. Vega-Menchaca, M.C., J. Verde-Star, A. Oranday-Cárdenas, M.E. Morales-Rubio, A. Núñez-González, M.A. Rivera-Guillen, L.B. Serrano-Gallardo and C. Rivas-Morales. (2013). Actividad antibacteriana y citotóxica de *Leucophyllum fruscescens* (Berl) I.M. Johnst del Norte de México contra *Staphylococcus aureus* de aislados clínicos. Rev. Mex. Cienc. Farm. 44(2):24-30.
64. Velaga, M.K., P.R. Yallapragada, D. Williams, S. Rajanna and R. Bettaiya. (2014). Hydroalcoholic seed extract of *Coriandrum sativum* (Coriander) alleviates lead-induced oxidative stress in different regions of rat brain. Biol. Trace Elem. Res. 159(1-3):351-63.
65. Wang, C., Y. Zhang, J. Liang, G. Shan, Y. Wang and Q. Shi. (2006). Impacts of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in testis. Clin. Chim. Acta. 370(1-2):82-88.
66. Wong, P.Y.Y. and D.D. Kitts. (2006). Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinumcrispum*) and cilantro (*Coriandrum sativum*) extracts. Food Chem. 97:505-515.
67. World Health Organization (WHO). 2002. Pautas generales para las metodologías de investigación y evaluación de la medicina tradicional.